A Glimpse of *Panax ginseng* Genome Structure Revealed from Ten BAC Clone Sequences Obtained by SMRT Sequencing Platform

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ABSTRACT Korean ginseng (*Panax ginseng*) is a well-known valuable medicinal plant with excellent therapeutic effects, however its complex genome structure has not been elucidated yet. To understand its genome structure, we obtained ten ginseng bacterial artificial chromosome (BAC) clone sequences by single-molecule real-time (SMRT) sequencing platform using a pooled DNA of the BAC clones. Out of the ten BAC clones, nine were completely assembled without any gap and one remained a single gap. The total length of BAC clone sequences was 1,163,364 bp. Sophisticated sequence analysis revealed that the 89.7% of the sequences are high copy repeat regions and the remaining 10.3% are non-repeat regions. Eleven protein-coding genes were identified in the non-repeat regions. Most of the repeat regions show more than 1,000 copies and complex structure of various repetitive elements. *Ty3/Gypsy* family long terminal repeat retrotransposons (LTR-RTs) are predominant repeats occupying 46.9% of the 1,163-kbp sequence. We identified six novel LTR-RTs and their insertion time. Fluorescence *in situ* hybridization (FISH) analysis demonstrated that *PgDel2* and *PgDel5* elements had a subgenome-biased distribution. Collectively, our analysis reveals that ginseng genome has very complex genome structure with abundant repetitive elements and rare gene frequency.

Keywords *Panax ginseng*, Bacterial artificial chromosome, SMRT sequencing, Long terminal repeat retrotransposon

INTRODUCTION

Korean ginseng (Asian ginseng, ginseng; *Panax ginseng* C.A. Meyer), which belongs to the Araliaceae family, is a slow-growing perennial herbal plant distributed mainly in Northeastern Asia (Yun 2001). It has been cultivated as an important medicinal crop for hundreds of years (Park *et al.* 2012a). Various types of ginsenosides biosynthesized in ginseng show numerous therapeutic effects on human, such as anti-wrinkle (So *et al.* 2008), anti-stress (Kumar *et al.* 2016), boosts the immune system (Quan *et al.* 2007),

controls symptoms of diabetes (Xie *et al.* 2005), Alzheimer's disease (Lee *et al.* 2007) and cancer (Wong *et al.* 2015). Despite its excellent pharmacological effects and many studies on efficacy of their medicinal components, breeding or genetic studies are still limited in this plant species. With increasing interests in the genome in recent years, various efforts have been made for marker development (Choi *et al.* 2011; Kim *et al.* 2012; Kim *et al.* 2013; Jung *et al.* 2014), DNA library construction (Bang *et al.* 2010; Hong *et al.* 2004) and complete assembly of 45S nuclear ribosomal DNA and chloroplast genome (Kim *et*

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al. 2015).

P. ginseng has an estimated genome size of over 3.5 Gbp for haploid genome equivalent (Hong *et al.* 2004; Waminal *et al.* 2012) and is regarded as an allotetraploid ($2n=4x=48$) (Choi *et al.* 2014). A previous study illustrated that *P. ginseng* genome had experienced two rounds of whole genome duplication (WGD) (Choi *et al.* 2013), and recent WGD occurring 2-3 million years ago (MYA), which made its genome much bigger than those of other diploid *Panax* species (Yi *et al.* 2004; Choi *et al.* 2013). Subsequently, studies were also carried out using simple sequence repeats derived from duplicated genes (Kim *et al.* 2014) and three repeat-rich bacterial artificial chromosome (BAC) clone sequences (Choi *et al.* 2014) to understand the genome structure of ginseng. However, characterization of more diverse long genome sequences is necessary for understanding of *P. ginseng* genome structure.

Recently, the genomes of various organisms have been analyzed rapidly and accurately with the advent of various high-throughput sequencing technologies (Pareek *et al.* 2011). Among those, the Pacific Biosciences (PacBio, Menlo Park, CA, USA) platform has gained attention in various genome projects (Gordon *et al.* 2016; Hoshino *et al.* 2016). This single molecule real-time (SMRT) sequencing platform has a unique feature that produce ultra-long read sequences, which allows to uncover the structure of complex repetitive elements dispersed in centromeres or telomeres of a genome (Bennett *et al.* 2016; Wolfgruber *et al.* 2016). In addition, this platform has also been widely used in genome assemblies of various organisms ranging from bacteria (Liao *et al.* 2015; Tanizawa *et al.* 2015) to complex eukaryotes with large and highly repetitive genomes (VanBuren *et al.* 2015; Ming *et al.* 2015; Gordon *et al.* 2016; Hoshino *et al.* 2016).

BAC clones harbor around 100-kb long DNA fragments derived from genome of a specific organism (O'Connor *et al.* 1989). Investigation and characterization of BAC sequences provide an estimated genome structure for the target organism. In this study, ten ginseng BAC clones were randomly selected and sequenced using SMRT sequencing platform. From the comprehensive analysis of assembled BAC sequences, genomic structures and novel long terminal repeat retrotransposons (LTR-RTs) were

characterized. This study will provide useful resources for further understanding of genome structure and evolution in *P. ginseng*.

MATERIALS AND METHODS

BAC clone selection and DNA extraction

Ten BAC clones were randomly selected from BAC library of *P. ginseng* cv. Chunpoong (Hong *et al.* 2004). The selected BAC clones were cultured in 500 mL 2xYT medium for 20 hours at 38°C and harvested by centrifugation. The BAC DNAs were isolated using the QIAGEN Plasmid Midi Kit (Qiagen, Hilden, Germany) according to a protocol suitable for very low-copy plasmid extraction. The extracted DNAs were quantified by NanoDrop ND-1000 (Thermo Scientific, USA).

SMRT sequencing and assembly

The same amount of each DNA sample of the ten BACs was pooled into a single tube and provided to DNA Link (Seoul, Korea) for the construction of a sequencing library. A 10-kb library was generated and sequenced using PacBio RS platform installed MagBead protocol. Two sequencing runs were performed using P4-C2 and P6-C4 chemistry versions. For production of long read, one SMRT cell was employed for each run. The generated reads were corrected and pre-assembled through the hierarchical genome assembly process (HGAP) method (Chin *et al.* 2013), and *de novo* assembly of BAC sequences was carried out using Celera assembler v8.2 (<http://wgs-assembler.sourceforge.net/>). Assembled sequences were polished by quiver program (<https://github.com/PacificBiosciences/GenomicConsensus>). Finally, the high-quality ten BAC sequences were obtained by removal of *E.coli* and vector sequences.

BAC sequences annotation

For preliminary prediction of genic regions, FGENESH program (Salamov and Solovyev 2000; <http://www.softberry.com/>) was used with tomato as reference model on default parameters. The gene structures were confirmed by BLASTP searches against the NCBI non-redundant (nr) protein sequence database and our customized trans-

criptome data. Repeat regions were predicted on the basis of NCBI Conserved Domain Search (CD-Search) (Marchler-Bauer *et al.* 2009; <https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) and CENSOR program (Kohany *et al.* 2006; <http://www.girinst.org/censor/>). Precise positions of repetitive elements in BAC sequences were finally determined by manual verification based on dot-plot analysis in which BAC sequences were compared with reported repeat elements of *P. ginseng* (Choi *et al.* 2014) using PIPMAKER (Schwartz *et al.* 2000; <http://pipmaker.bx.psu.edu/cgi-bin/pipmaker?advanced>) and Tandem Repeat Finder (Benson 1999; <http://tandem.bu.edu/trf/trf.html>) programs with default parameters.

Repeat element analysis

To classify the repetitive elements, reverse transcriptase (RT) domain of each LTR-RT was extracted from BAC sequences and compared with core database of GyDB (Llorens *et al.* 2011; http://gydb.org/index.php/Main_Page) using BLASTX searches. Internal structure of LTR-RTs was confirmed by CD-Search analysis. To estimate insertion time of LTR-RTs, each LTR sequence from both sides were extracted and aligned using ClustalW (Thompson *et al.* 1994) with default parameters. The numbers of transition (Ts) and transversion (Tv) mutations were calculated using MEGA version 7.0 (Kumar *et al.* 2016). The pairwise distance between the LTR sequences from both side was calculated using the Kimura two-parameter model (Kimura 1980). A substitution rate value was adopted as 1.22×10^{-8} which was used in the previous expressed sequence tag analysis in ginseng (Choi *et al.* 2013).

WGS read mapping and genome structure analysis

Whole genome sequence (WGS) reads from *P. ginseng* cv. Chunpoong were generated using HiSeq 2000 (Illumina, Inc., San Diego, CA, USA) as a part of *P. ginseng* genome sequencing project (data not shown). The 30 Gbp reads corresponding to approximately 10 times of ginseng genome size were randomly sampled and mapped onto assembled BAC sequences by CLC Assembly Cell version 4.21 software (<https://www.qiagenbioinformatics.com/products/clc-assembly-cell/>) with default parameters. The

genome proportion of LTR-RTs was calculated by dividing the total mapping depth of each LTR-RT region by 30 Gbp. To estimate repetitive regions of the BAC sequence, RepeatMasker software (<http://www.repeatmasker.org>) was used with default parameters using a custom repeat database comprising both repeat sequences identified in our previous study (Choi *et al.* 2014) and newly in this study.

Fluorescence *in situ* hybridization (FISH) analysis

PgDel5 FISH probe was designed using its RT domain sequences (forward: 5'-TACCAATCTTCAGTGACTT-ACACGA-3', reverse: 5'-TCGAGTTATAAATTGTGC-GTAATGA-3') and *PgDel2* FISH probe which showed subgenome-specific pattern was used from a previous study (Choi *et al.* 2014) for FISH analysis. PCR amplicons were labeled through direct nick-translation with Alexa Fluor® 488-5-dUTP (Invitrogen, C11397) and Texas Red-5-dUTP (Perkin Elmer, NEL417001EA), respectively. FISH procedures were as described previously (Waminal *et al.* 2012). Briefly, FISH immediately followed fixation of slides with somatic metaphase chromosomes in 4% paraformaldehyde without pepsin and RNase pretreatment. The hybridization mixture contained 50% formamide, 10% dextran sulfate, 2×SSC, 5 ng/μL salmon sperm DNA and 20 ng/μL of each probe DNA and was adjusted with DNase- and RNase-free water (Sigma, USA, #W4502) to a total volume of 40 μL/slide. The hybridization mixture was denatured at 90°C for 10 minutes and immediately kept on ice for at least 5 minutes prior to mounting on slides. After covering with a glass coverslip, the chromosomes were denatured at 80°C for 3-5 minutes on a hot plate. The slides were then immediately transferred into a humid chamber preset at 37°C and incubated overnight (~16 hours). The following day, the slides were washed in 2× SSC (15 minutes at RT), 0.1× SSC (35 minutes at 42°C), and finally 2× SSC (30 minutes at room temperature). Images were captured with an Olympus BX53 fluorescence microscope equipped with a Leica DFC365 FS CCD camera, and processed using Cytovision ver. 7.2 (Leica Microsystems, Germany). Further image enhancements were performed using Adobe Photoshop CC.

RESULTS

Sequencing and assembly of BAC clones

Ten BAC clones were randomly selected and pooled for sequencing using PacBio SMRT sequencing platform. Two independent reactions were conducted using P4-C2 and P6-C4 chemistry. Among two sequencing reactions, P4-C2 chemistry produced 44,736 reads with an average length of 3,605 bp, while P6-C4 chemistry produced 100,844 reads with an average length of 4,813 bp (Table 1). Approximately, 9.7% of reads were longer than 10 kb. Short and low quality reads (<50 bp and <0.75 respectively) were removed for optimal assembly. Initial assemblies using each read set from P4-C2 and P6-C4 chemistries produced good quality of contig sequences with only seven gaps and one gap, respectively. By combining of both data, we obtained complete sequences without gap for nine BACs (BAC IDs 8L14, 9P08, 6B09, 8G13, 5N01, 8P22, 8C22, 5B21 and 10M13) and with one

gap for BAC ID 7P20. Overall, a total 1,163,364 bp of assembled sequences were generated (Table 2).

Sequence annotation

Annotations of ten BAC clone sequences allow us to predict complex genome structure of ginseng, which is mainly composed of various repetitive components with relatively small portion of genic regions (Fig. 1). The six BAC sequences (BAC IDs 8L14, 8G13, 5N01, 8P22, 8C22, and 5B21) harbor only repeat elements without any gene. Among repeat elements, *Ty3/Gypsy* type LTR-RTs were predominantly distributed throughout the entire sequences. Eleven genes were identified in four BAC sequences (BAC IDs 9P08, 6B09, 10M13 and 7P20) which have less repetitive elements (Fig. 1, Table 3). In addition, six novel LTR-RTs were identified in four BAC sequences (BAC IDs 8G13, 8P22, 8L14, 7P20 and 10M13) and one tandem repeat (TR) was identified in the middle of BAC ID 5N01 sequence (Table 4).

Table 1. Summary of sequence statistics.

Read size	Sequencing chemistry					
	P4-C2			P6-C4		
	Total read length (bp)	Number of reads	Average read length (bp)	Total read length (bp)	Number of reads	Average read length (bp)
~5 kb	7,107,7597	33,924	2,095	160,132,596	66,268	2,416
5 kb~10 kb	58,026,397	8,263	7,022	164,293,017	22,992	7,145
~10 kb	32,183,323	2,549	12,625	161,018,656	11,584	13,900
Total	161,287,317	44,736	3,605	485,444,269	100,844	4,814

Table 2. Assembly statistics of ten BAC clones.

BAC ID	Assembled length (bp)	Number of contigs			Non-repetitive region (bp, <50 mapping depth)	GenBank Accession number
		P4-C2	P6-C4	Final		
8L14	173,429	5	1	1	11,843	KY513616
9P08	139,247	1	1	1	7,290	KY513618
6B09	131,717	2	1	1	25,544	KY513612
8G13	123,350	1	1	1	902	KY513615
5N01	117,096	2	1	1	90	KY513611
8P22 ²⁾	109,113	1	-	1	232	KY513617
8C22	107,984	1	1	1	61,448	KY513614
5B21	100,349	1	1	1	196	KY513610
10M13 ²⁾	97,432	1	-	1	2,611	KY513619
7P20	63,647	2	2	2	9,707	KY513613

²⁾Two BAC clones, 8P22 and 10M13, were not included in the second sequencing run using P6-P4 chemistry.

Fig. 1. Sequence analysis of ten BAC clones in ginseng. Horizontal black bars represent ten BAC sequences. Identified genome components were shown above the black bar and depicted according to their position on the BAC sequences. The description of each components is shown at the bottom right. Graphs under the black bars represent the depth distribution of 30 Gbp WGS read mapping (approximately 10x coverage). RD indicate the read depth plot for each nucleotide position using 10x WGS.

Table 3. Annotation summary of identified eleven genes in BAC sequences.

Gene annotation based on BLASTP searches	BAC ID	Position (bp)	# of exon	Accession no. (E-value)
Cellulose synthase A catalytic subunit 3 [UDP-forming]	9P08	215-9,773	14	XP_017226278.1 (0.0)
Calcium-dependent protein kinase 28	6B09	1-5,383 (partial)	9	XP_017251289.1 (0.0)
Uncharacterized protein	6B09	18,057-19,415	3	XP_017251291.1 (8e-115)
Transformation/transcription domain-associated protein	6B09	21,538-59,489	35	XP_017217620.1 (0.0)
Transformation/transcription domain-associated protein	6B09	94,283-120,564	35	XP_017217620.1 (0.0)
Acyl carrier protein 1	10M13	1,127-3,315	4	XP_017257036.1 (1e-55)
Superoxide dismutase	10M13	33,367-36,315	7	O22668.1 (1e-100)
Bifunctional 3-dehydroquinate dehydratase/ shikimate dehydrogenase	10M13	44,804-50,967	10	XP_017220070.1 (0.0)
Uncharacterized protein	10M13	55,540-58,599	1	XP_017218710.1 (0.0)
Uncharacterized protein	7P20	2,245-7,217	5	XP_017238997.1 (0.0)
Protein FAR1-related sequence 5-like	7P20	53,287-55,512	2	XP_015866013.1 (0.0)

Table 4. List of novel LTR retrotransposons identified in BAC sequences.

Type	BAC ID	Position (bp)	TSD ²⁾	Length / LTR length (bp)		Ts ^{y)}	Tv ^{x)}	Ts/Tv	K ^{w)}	Insertion time ^{v)} (MYA)
<i>PgDel4</i>	8G13	75,443-76,688, 77,213-87,016	GCAAC	11,050	Left 2,542 Right 2,551	21	15	1.4	0.014	0.57
<i>PgDel5</i>	8P22	1-10,587	CAAGC	10,587	Left 1,332 Right 3,522	5	8	0.63	0.010	0.41
<i>PgDel6</i>	8P22	20,535-32,786	GCGCT	12,252	Left 3,195 Right 3,195	60	53	1.13	0.037	1.51
<i>PgTork2</i>	8L14	140,265-148,881	GCAAC	8,615	Left 1,544 Right 1,544	13	6	2.17	0.012	0.49
<i>PgSire1</i>	7P20	32,485-35,143, 39,282-46,836	AAAGG	10,214	Left 1,558 Right 1,554	41	14	2.93	0.037	1.51
<i>PgSire2</i>	10M13	65,051-65,495, 65,765-71,239	CCAGT	5,920	Left 251 Right 253	8	3	2.67	0.045	1.81

²⁾Target site duplication.

^{y)}Number of transition mutations.

^{x)}Number of transversion mutations.

^{w)}Kimura's distance.

^{v)}Insertion times were estimated by adopting the substitution rate of 1.22×10^{-8} .

Classification and characterization of LTR-RTs

Among the six novel LTR-RTs, three are classified to *Del* family, which belong to *Ty3/Gypsy* type LTR-RTs, and named as *PgDel4*, *PgDel5*, and *PgDel6* subfamilies. The remaining three elements had RT domain similar to *Ty1/Copia* type LTR-RTs. Two belonged to *Sire* family, named as *PgSire1* and *PgSire2*, and another one belong to *Tork* family, named as *PgTork2*. The overall structure of

each element was confirmed by CD-Search (Fig. 2). Unlike the previously reported *PgDel* subfamilies (*PgDel1*, 2, 3), a zinc-knuckle (accession number cl15298 in NCBI CDD) and a chromodomain (accession number cl15261 in NCBI CDD) were not found in the *PgDel4* and *PgDel6*. On the other hand, *PgTork2* contain an unusual zinc-knuckle domain, which is a distinct feature from other *Ty1/Copia* family members. *PgSire1* seemed to experience internal domain losses caused by the insertion of other LTR-RT

Fig. 2. Structure of newly identified long terminal repeat retrotransposons (LTR-RTs) in ginseng BAC sequences. Vertical bars on both ends represent the target site duplication (TSD). The mark (+ or -) next to the transposable elements ID indicates the direction on the BAC sequence. Identified internal domain, AP (aspartic protease), CH (chromodomain), GAG (capsid protein), INT (integrase), RH (RNase H), RT (reverse transcriptase) and Zn (zinc knuckle), were depicted according to their position in the internal regions. The dotted box region of *PgDel5* are estimated structure because it was truncated in the BAC clone sequence. The omitted region of *PgSire1* indicates area lost by external factors.

members belonged to *Ty3/Gypsy* family.

To estimate insertion time of the six novel LTR-RTs, the ratios of transition to transversion (Ts/Tv) was calculated between both flanking LTR sequences (Table 4). The Ts/Tv ranged from 0.63 to 2.93 with an average value of 1.82. We measured nucleotide substitution rate between both LTRs of each element to estimate insertion time of each of six LTR-RTs. Kimura's nucleotide substitution rates ranged from 0.010 to 0.045 and the insertion time of each element was estimated to 0.41-1.81 MYA.

Estimation of ginseng genome structure

Mapping of 10x WGS reads revealed that approximately 10% of BAC sequences were non-repetitive and the remaining 90% regions were repeat-replete regions (Table 2, Fig. 1). The non-repetitive regions showed less than 50x coverage mapping depth, while repeat-replete regions showed high mapping depth with more than 10,000x

coverage. The repetitive regions were mainly occupied by various LTR-RTs with complex and nested insertion patterns. For calculation of actual repeat proportion in the ten BACs, we conducted repeat masking with previously reported and newly identified LTR-RTs. Homology-based search revealed that over 60% of the BAC sequences comprised various LTR-RTs (Table 5). Most of the regions were occupied by *Ty3/Gypsy* type LTR-RTs family members (46.88%), unknown repeats (8.57%) and *Ty1/Copia* family members (5.09%). *PgDel* elements are the most common in the BAC sequences, of which, *PgDel1* was the most abundant (24.17%).

We estimated the proportion of LTR-RTs in whole ginseng genome by mapping 30 Gbp of WGS reads onto each element. Approximately 36% of the ginseng genome was estimated to be occupied by 14 LTR-RTs including eight reported and six novel subfamilies. The estimated genome proportion of LTR-RTs is lower than the

Table 5. Proportion of LTR-RTs in the ginseng genome calculated from repeat masking and WGS read mapping.

Type	Proportion in BACs (%)	Expected proportion in genome (%)
<i>Ty3/Gypsy</i>	46.88	28.66
<i>PgDel</i>	34.53	24.04
<i>PgDel1</i>	24.17	20.0
<i>PgDel2</i>	0.72	0.93
<i>PgDel3</i>	4.07	1.17
<i>PgDel4</i>	1.08	0.37
<i>PgDel5</i>	1.61	0.51
<i>PgDel6</i>	2.88	1.06
<i>PgTat</i>	11.13	4.1
<i>PgTat1</i>	9.83	3.85
<i>PgTat2</i>	1.3	0.25
<i>PgAthila</i>	1.22	0.52
<i>Ty1/Copia</i>	5.09	5.1
<i>PgTork</i>	2.32	1.28
<i>PgTork1</i>	0.9	0.72
<i>PgTork2</i>	1.12	0.56
<i>PgSire</i>	2.9	1.2
<i>PgSire1</i>	2.25	0.92
<i>PgSire2</i>	0.65	0.28
<i>PgOryco</i>	0.17	0.04
Degenerated LTR-RTs	8.57	2.58
Total	60.54	36.34

proportion found in the BAC sequences. However, both results show similar distribution pattern. For example, both result show that *PgDel1* members are most abundant although the estimated value for genome proportion (20.0%) is lower than the proportion in the 1,163 Kb sequences (24.2%) (Table 5).

Our previous FISH analysis revealed that *PgDel1* was present in all chromosomes and *PgDel2* was in 12 of 24 chromosomes (Choi *et al.* 2014). To understand the chromosomal distribution of repeat elements, two LTR-RTs, *PgDel2* and *PgDel5* showing uneven distribution were selected for cytogenetic analysis. Simultaneous FISH analysis with *PgDel2* and *PgDel5* displayed biased signal between twelve opposite chromosome pairs (Fig. 3). *PgDel2* (green signal) was present in only twelve chromosome pairs as consistent with previous study (Choi *et al.* 2014), while *PgDel5* (red signal) showed intense signal in other twelve *PgDel2*-poor chromosome pairs.

Fig. 3. The FISH analysis of *PgDel2* (green signals) and *PgDel5* (red signals) on somatic metaphase chromosomes. Bar, 5 μ m.

DISCUSSION

Sequencing of pooled BAC clones using SMRT sequencing

The sensational development of sequencing technology enabled assembly of nearly complete genomes in various species (Pareek *et al.* 2011). SMRT sequencing produce long contiguous sequences, which are essential for comprehensive assembly of repeat-rich genomes. It is also well suited for assembly of relatively small genomes such as bacterial and organellar genomes or BAC sequences (Frank *et al.* 2015). With this scientific blessing, we successfully assembled ten ginseng BAC clones with abundant repetitive elements. In this study, we conducted single-cell SMRT sequencing for a ten BAC clone pool using two different chemistries, P4-C2 and P6-C4 chemistries. Although we report ten BAC sequences finished by combining of both reactions, it should be noted that each single reaction produced almost complete assembly which can be utilized for further genome study.

Overview of a ginseng genome structure

The ten randomly selected BAC clone sequences showed brief overview of a ginseng genome. Among the

ten BACs, two BACs, 6B09 and 10M13, contained four genes with less repetitive elements, two BACs, 7P20 and 9P08, contained two and one gene, respectively, with moderate repetitive elements, whereas the other six BACs were entirely composed of only repetitive elements without any gene. In particular, many LTR-RTs occupied the largest portion of the BAC sequences with complex nested insertion patterns (Table 5, Fig. 1). The results suggest that LTR-RTs might play a major role in the increase of the genome size such as many other plants with large genomes size (SanMiguel *et al.* 1996; Park *et al.* 2012b).

Our previous study identified eight LTR-RTs which occupied a third of the ginseng genome (Choi *et al.* 2014). Here, we characterized six novel LTR-RTs. Overall, a total of 14 LTR-RTs were characterized from 13 ginseng BAC sequences. More than 60% of the whole BAC sequences were characterized as components of 14 LTR-RTs by repeat masking. Although the 14 LTR-RTs are major repeats occupying more than 60% of ginseng genome, other uncharacterized LTR-RT members, transposable elements and repetitive elements will be found in the ginseng genome.

We estimated the copy numbers of all the components in the 1,163 kb sequences by mapping of 10x WGS reads on the homologous regions based on more than 80% sequence similarity. Approximately 10% of BAC sequences were non-repetitive, while, the remaining 90% show repeat regions with more than 10,000x coverage from the 10x WGS. Overall, 90% of the 1,163 kb are composed of various repetitive elements with over 1,000 copies in the ginseng genome. Repeat masking tool revealed that 60% of 1,163 kb sequences are components of 14 LTR-RTs. However, 36.34% of WGS reads were mapped on the 14 LTR-RTs that is much lower than the proportion of LTR-RTs (60%) occupying in the 1,163 kb sequences (Table 5) that is similar phenomena found in our previous research (Choi *et al.* 2014). More diverse forms of LTR-RT members will be in the actual ginseng genome that make biased genome proportion estimation values. However, both analysis show the relative abundance for each family member. The most abundant LTR-RTs are *PgDel* and *PgTat* families. Genome proportion of *PgDel* and *PgTat* families are estimated to be 34.5-24.0% and 11.1-4.1%,

respectively (Table 5).

Amplification of LTR-RTs during ginseng genome evolution

We proposed that recent WGD in ginseng genome was caused by an allotetraploidization event based on finding of subgenome-specific distribution of transposable elements, *PgDel2* (Choi *et al.* 2013, 2014). The subgenome-specific LTR-RTs distribution was also discovered in other plants such as allopolyploid wheat (Sabot *et al.* 2006; Salina *et al.* 2011). In this study, we found another clue for allopolyploidization event in the ginseng genome. FISH analysis revealed that rich signal of *PgDel5* are located on 12 *PgDel2*-poor chromosome pairs. We estimated that allotetraploidization event in ginseng is originated from the hybridization of two related ancestral species, as occurred in other allotetraploid plant species. The ancestral species is unknown for *P. ginseng* up to now. Discovery of these sub-genome unique LTR-RTs may contribute to unveil the evolutionary story by further comparative analysis against related *Panax* species.

Insertion time of the LTR-RT has been calculated by estimating the sequence divergence between two LTRs that independently accumulate point mutations at each LTR sequences (Dangel *et al.* 1995; SanMiguel *et al.* 1996; SanMiguel *et al.* 1998). The insertion time of six novel LTR-RTs was estimated to be 0.41-1.81 MYA (Table 4). These results also supported a hypothesis that speciation of ginseng from related *Panax* species might be accelerated by uneven amplification of various transposable elements after WGD.

The structure and characteristic of the 1,163 kb ginseng genome sequences analyzed in this study will expand our understanding about the complex genome structure of ginseng. Furthermore, our data provide valuable resources for understanding of genome structure and evolution, as well as for breeding and related researches in the genus *Panax*.

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